# A new 5α,8α-epidioxy sterol from the Okinawan marine sponge of the *Axinyssa* genus

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*A new sterol (axinysterol) was isolated from the Okinawan marine sponge of the genus* Axinyssa. *The structure of axinysterol was assigned as 5α,8α-epidioxyergosta-6,22,25-trien-3β-ol based on spectroscopic analysis and chemical transformation.* (*Steroids* **58:**410–413, 1993)

**Keywords:** 5α,8α-epidioxyergosta-6,22,25-trien-3β-ol; axinysterol; marine sponge; *Axinyssa* genus; sterol; structure

## Introduction

Although a large number of sterol structures have been found in the study of marine organisms, 5α,8α-epidioxy sterols are limited.[1–6] In the course of our investigations[7–9] on chemical substances from Okinawan marine invertebrates, we isolated a new 5α,8α-epidioxy sterol, axinysterol **(1)**, from the sponge (genus *Axinyssa*). The structure of axinysterol was determined based on spectroscopic analysis and chemical transformation. Axinysterol **(1)** is the first example of a 5α,8α-epidioxy sterol with a 24-methyl-22,25-diene system in the side chain.

## Experimental

### *General method*

Melting points are uncorrected. Optical rotations were measured in $CHCl_3$ solution on a JASCO DIP-360 automatic polarimeter. $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AM-400 spectrometer in $CDCl_3$. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26 ppm). $^{13}C$ NMR spectra were referenced to the center peak of $CDCl_3$ at 77.1 ppm. The multiplicities of $^{13}C$ resonances were achieved by DEPT experiments which were performed using polarization transfer pulses of 90° and 135°, first obtaining only signals for CH groups and then positive signals for CH and $CH_3$ and negative signals for $CH_2$ groups. Infrared (IR) spectra were recorded with a Perkin-Elmer FT-IR 1710 spectrophotometer. High-resolution mass spectra (HRMS) were obtained by electron impact on a Hitachi M-80 spectrometer. Column chromatography was carried out on Fuji-Davison Silica Gel BW-820 MH (70-200 mesh).

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Received October 9, 1992; accepted April 23, 1993.

### *Extraction and isolation procedure*

Specimens of genus *Axinyssa* were collected from the coral reef of Ishigaki Island (Okinawa, Japan) at a depth of about 5 m, during October, 1988. The sponge was identified by Professor R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam. Reference specimens are deposited in his collection (voucher number: ZMA POR. 9343).

The wet specimens (200 g) were cut into small sections and extracted with methanol (200 ml, twice) at room temperature. The extracts were concentrated under reduced pressure to give a residue (7.4 g), which was partitioned between water and ethyl acetate. The ethyl acetate-soluble portion was concentrated under reduced pressure to give a residue (1.1 g), a part of which (0.79 g) was chromatographed on a silica gel column (20 g), and the column was eluted first with hexane/ethyl acetate (10 : 1, 160 ml) and then with hexane/ethyl acetate (5 : 1, 120 ml). The fraction (159 mg) eluted with hexane/ethyl acetate (5 : 1) was shown to include axinysterol as a major component from its $^1H$ NMR spectrum. The fraction was further subjected to repeated silica gel column chromatography to give a fraction (37 mg) whose $^1H$ NMR spectrum showed that about 90% amount of axinysterol was present in this fraction. For further purification, crude axinysterol was converted to *p*-bromobenzoyl ester as follows.

### p-*Bromobenzoylation of crude axinysterol*

*p*-Bromobenzoyl chloride (60 mg) was added to a solution of crude axinysterol (**1**, 37 mg) in pyridine (1.5 ml). After being stirred at room temperature overnight, the reaction mixture was diluted with ether. The ethereal solution was washed with water, saturated $CuSO_4$ solution, water, saturated $NaHCO_3$ solution, and again with water. The ethereal solution was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a residue (65 mg), which was chromatographed on a silica gel column (7 g). The column was eluted with hexane/$CHCl_3$ (1 : 1) to give crude *p*-bromobenzoate (**2**, 40 mg). Purification was conducted by HPLC (silica gel, hexane/$CHCl_3$/ethyl acetate = 40 : 10 : 1 as an eluent, UV 254 nm) to give axinysterol *p*-bromobenzoate (**2**, 22 mg). Colorless crystals, mp 180–188 C.

**Table 1** $^{13}C$ and $^{1}H$ NMR data for axinysterol **(1)** and axinysterol *p*-bromobenzoate **(2)**[a]

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$(mult.) | $\delta_H$(mult., J in Hz) | $\delta_C$(mult.) | $\delta_H$(mult., J in Hz) |
| 1 | 34.8 (t) | | 34.4 (t) | |
| 2 | 30.2 (t) | | 26.4 (t) | |
| 3 | 66.6 (d) | 3.95 (m) | 70.5 (d) | 5.24 (m) |
| 4 | 37.1 (t) | 2.11 (ddd, 1.9, 5.1, 13.7) | 33.3 (t) | 2.16 (dd, 11.8, 13.6, β-H)<br>2.26 (ddd, 1.6, 5.4, 13.6, α-H) |
| 5 | 82.3 (s) | | 81.8 (s) | |
| 6 | 135.4 (d)[b] | 6.24 (d, 8.4) | 135.1 (d) | 6.26 (d, 8.5) |
| 7 | 130.8 (d) | 6.50 (d, 8.4) | 131.0 (d) | 6.53 (d, 8.5) |
| 8 | 79.5 (s) | | 79.4 (s) | |
| 9 | 51.2 (d) | | 51.1 (d) | 1.52 (m) |
| 10 | 37.1 (s) | | 37.1 (s) | |
| 11 | 20.7 (t)[c] | | 20.7 (t)[b] | |
| 12 | 39.5 (t) | | 39.4 (t) | 1.97 (dd, 2.5, 9.2)<br>1.25 (m) |
| 13 | 44.7 (s) | | 44.6 (s) | |
| 14 | 51.8 (d) | | 51.6 (d) | 1.60 (m) |
| 15 | 23.5 (t)[c] | | 23.4 (t)[b] | |
| 16 | 28.7 (t)[c] | | 28.6 (t)[b] | |
| 17 | 56.3 (d) | | 56.2 (d) | 1.25 (m) |
| 18 | 13.0 (q) | 0.82 (s) | 12.9 (q) | 0.83 (s) |
| 19 | 18.3 (q) | 0.88 (s) | 18.1 (q) | 0.95 (s) |
| 20 | 39.6 (d) | | 39.5 (d) | |
| 21 | 20.69 (q)[d] | 1.00 (d, 6.6) | 20.6 (q)[c] | 1.01 (d, 6.6) |
| 22 | 135.5 (d)[b] | 5.23 (dd, 7.2, 15.2) | 135.3 (d) | 5.24 (dd, 7.2, 15.2) |
| 23 | 132.0 (d) | 5.26 (dd, 6.3, 15.2) | 132.0 (d) | 5.27 (dd, 6.3, 15.2) |
| 24 | 43.7 (d) | 2.71 (quintet, 6.5) | 43.6 (d) | 2.72 (quintet, 6.5) |
| 25 | 149.8 (s) | | 150.0 (s) | |
| 26 | 109.0 (t) | 4.69 (br s) | 108.9 (t) | 4.70 (br s) |
| 27 | 20.74 (q)[d] | 1.67 (br s) | 20.7 (q)[c] | 1.68 (s) |
| 28 | 18.9 (q) | 1.08 (d, 6.9) | 18.9 (q) | 1.08 (d, 6.9) |
| 1′ | | | 127.8 (s) | |
| 2′ | | | 131.1 (d) | 7.88 (d, 8.6) |
| 3′ | | | 131.6 (d) | 7.56 (d, 8.6) |
| 4′ | | | 129.6 (s) | |
| CO | | | 164.9 (s) | |

[a] Assignments and correlations of $^{1}H$ and $^{13}C$ signals were made based on two-dimensional $^{13}C$-$^{1}H$ COSY and $^{13}C$-$^{1}H$ long-range correlation spectra.
[b,c,d] Values with the same superscript may be interchanged in each column.

$[\alpha]_D$ +15.2° (*c* 0.3, $CHCl_3$). HRMS, m/z (assignment, relative intensity) 408.3037 ($M^+ - BrC_6H_4CO_2H$, $C_{28}H_{40}O_2$ requires 408.3026, 11). $^{1}H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) are listed in Table 1.

## *Hydrolysis of axinysterol* p-*bromobenzoate*

Potassium carbonate (5 mg) was added to a solution of axinysterol *p*-bromobenzoate (**2**, 3 mg) in MeOH (0.5 ml), and the mixture was stirred at room temperature overnight. After diluting the reaction mixture with water, it was extracted with ether. The ethereal solution was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/$CHCl_3$/ethyl acetate = 2 : 2 : 1 as an eluent) to give axinysterol (**1**, 1 mg). Colorless crystals, mp 114–116 C.$[\alpha]_D$ −7.7° (*c* 0.16, $CHCl_3$). EIMS, m/z (assignment, relative intensity), 426 ($M^+$, 6), 408 ($M^+ - H_2O$, 13), 394 ($M^+ - O_2$, 66). HRMS, m/z (assignment), 408.3008 ($M^+ - H_2O$, $C_{28}H_{40}O_2$ requires 408.3026). IR (KBr) 3440, 2954, 2869, 1644, 1455, 1378, 1044, 936, 890 $cm^{-1}$. $^{1}H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) are listed in Table 1.

After having obtained pure axinysterol, we reexamined the remaining crude ethyl acetate soluble portion and found that axinysterol was indeed present.

## *Oxidation of ergosterol*[10]

Oxygen was passed through a solution of ergosterol (100 mg) in 95% ethanol containing methylene blue (0.2 mg) as a sensitizer under the irradiation of tungsten lamp (40 W) for 1 hour. The reaction mixture was concentrated under reduced pressure. The residue thus obtained was chromatographed on a silica gel column (hexane/ethyl acetate = 2 : 1 as an eluent) to give 5α,8α-epidioxyergosta-6,22-dien-3β-ol (ergosterol peroxide, **3**, 85 mg).

## *Catalytic hydrogenation of ergosterol peroxide*

A mixture of ergosterol peroxide (10 mg) and 10% palladium charcoal (10 mg) in ethyl acetate (1 ml) was vigorously stirred at room temperature under a hydrogen atmosphere for 2 hours. The reaction mixture was filtered through a Celite column and the filtrate concentrated under reduced pressure. The residue was chromatographed on a silica gel column ($CH_2Cl_2$/methanol = 60 : 1 as an eluent) to give ergost-7-ene-3β,5α-diol[11] (**4**, 7 mg). Colorless crystals, mp 218–228 C. $[\alpha]_D$ +14.3° (*c* 0.68, $CHCl_3$). MS, m/z (assignment, relative intensity) 398 ($M^+ - H_2O$, 30), 383 ($M^+ - H_2O - CH_3$, 11), 365 ($M^+ - 2H_2O - CH_3$, 32). $^{1}H$ NMR (400 MHz, $CDCl_3$) δ(ppm) 0.55 (3H, s), 0.78 (3H, d, *J* = 6.8 Hz), 0.79 (3H, d, *J* = 6.8 Hz), 0.86 (3H, d, *J* = 6.9 Hz), 0.92

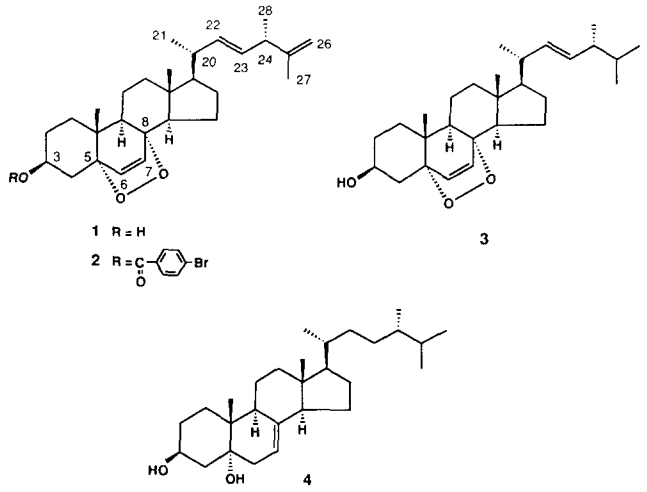


**Figure 1** Structure of axinysterol (**1**) and related compounds.

(3H, s), 0.93 (3H, d, $J$ = 6.5 Hz), 4.04 (1H, m), 5.07 (1H, m). $^{13}$C NMR (100 MHz, $CDCl_3$) δ(ppm) 12.0 (q), 15.6 (q), 17.8 (q), 18.7 (q), 19.1 (q), 20.6 (q), 21.7 (t), 23.0 (t), 28.0 (t), 30.8 (t), 31.1 (t), 31.6 (d), 31.7 (t), 33.8 (t), 36.7 (d), 37.1 (t), 38.0 (s), 39.2 (d), 39.7 (t), 42.9 (t), 43.9 (s), 44.1 (d), 55.0 (d), 56.2 (d), 67.6 (d), 74.3 (s), 114.4 (d), 140.9 (s).

### *Catalytic hydrogenation of axinysterol*

Axinysterol (**1**, 4 mg) was hydrogenated under conditions similar to those for ergosterol peroxide to give ergost-7-ene-3β,5α-diol (**4**, 2 mg). Colorless crystals, mp 209–215 C. $[\alpha]_D$ +14.9° ($c$ 0.22, $CHCl_3$). The $^1$H NMR spectrum (400 MHz, $CDCl_3$) of the diol was identical to that of the diol **4** from ergosterol peroxide.

## Results and discussion

The methanol extract obtained from genus *Axinyssa* was partitioned between ethyl acetate and water. The ethyl acetate soluble portion was repeatedly chromatographed on a silica gel column to give crude axinysterol. For further purification, crude axinysterol was converted to *p*-bromobenzoate **2** which was purified by HPLC. Hydrolysis of **2** by methanolic potassium carbonate gave axinysterol (**1**) (Figure 1).

The molecular formula of $C_{28}H_{42}O_3$ for axinysterol (**1**) was determined by HRMS measurement. All 28 carbons appeared in the $^{13}$C NMR spectrum and DEPT indicated the presence of five methyls, seven $sp^3$ methylenes, one $sp^2$ methylene, six $sp^3$ methines, four $sp^2$ methines, four $sp^3$ quaternary carbons and one $sp^2$ quaternary carbon. Table 1 shows $^{13}$C and $^1$H correlations in comparison to the results obtained by examination of the two-dimensional (2D) $^{13}$C-$^1$H COSY spectrum of *p*-bromobenzoate **2**. The IR spectrum showed absorption at 3440 $cm^{-1}$ due to a hydroxyl group. The $^1$H NMR signal at δ 3.95 (m, H-3) ppm showed this hydroxyl group to be secondary. This signal shifted down field to δ 5.20–5.31 (m) ppm in its *p*-bromobenzoate **2**. The presence of a peroxy group was suggested by the mass fragment ion of m/z 394 from the molecular ion (m/z 426) by loss of an oxygen molecule, and was also suggested by $^{13}$C NMR signals [δ 79.5 (s, C-8), 82.3 (s, C-5)] due to quaternary carbons each bearing an oxygen atom of the peroxy group. The presence of three carbon-carbon double bonds (one exo olefin and two disubstituted olefins) was indicated by the $^1$H and $^{13}$C NMR spectra; $\delta_H$ 4.69 (2H, br s, H-26), 5.23 (1H, dd, $J$ = 7.2, 15.2 Hz, H-22), 5.26 (1H, dd, $J$ = 6.3, 15.2 Hz, H-23), 6.24 (1H, d, $J$ = 8.4 Hz, H-6), 6.50 (1H, d, $J$ = 8.4 Hz, H-7), $\delta_C$ 109.0 (t, C-26), 130.8 (d, C-7), 132.0 (d, C-23), 135.4 (d, C-6 or C-22), 135.5 (d, C-6 or C-22), and 149.8 (s, C-25). The $E$ configuration of one of the two disubstituted olefins was shown by the coupling constant ($J$ = 15.2 Hz) between the olefinic protons at 5.23 and 5.26 ppm. The $Z$ configuration of another disubstituted olefin was also shown by the coupling constant ($J$ = 8.4 Hz) between the olefinic protons at 6.24 and 6.50 ppm. The low field shifts of these olefinic protons suggested this olefin and the above-mentioned peroxy group to constitute a 1,2-dioxacyclohex-4-ene system.

The $^{13}$C-$^1$H long-range correlation spectrum of **2** showed the partial structures of the B ring and side chain. As shown in Figure 2, correlations of H-6/C-5, H-6/C-8, H-7/C-5, H-7/C-8, H-9/C-8, H-19/C-5, H-19/C-9, and H-19/C-10 indicated the presence of a 7-methyl-2,3-dioxabicyclo[2.2.2]oct-5-ene system in the B ring. Correlations of H-21/C-20, H-21/C-22, H-27/C-24, H-27/C-25, H-28/C-23, H-28/C-24, and H-28/C-25 demonstrated the presence of a 2,3-dimethylhepta-1,4-dienyl group in the side chain. Other correlations observed are also shown in Figure 2.

The above findings indicated the plane structure of axinysterol to be 5,8-epidioxyergosta-6,22,25-trien-3-ol. The following chemical transformations established the full structure of **1** including absolute stereochemistries. The catalytic hydrogenation of 5α,8α-epidioxyergosta-6,22-dien-3β-ol (ergosterol peroxide, **3**), prepared by photosensitized oxygenation of ergosterol,[10] in the presence of 10% palladium charcoal gave a diol (**4**, mp 218–228 C, $[\alpha]_D$ +14.3°).[11,12] Similar catalytic hydrogenation of axinysterol (**1**) gave a diol (mp

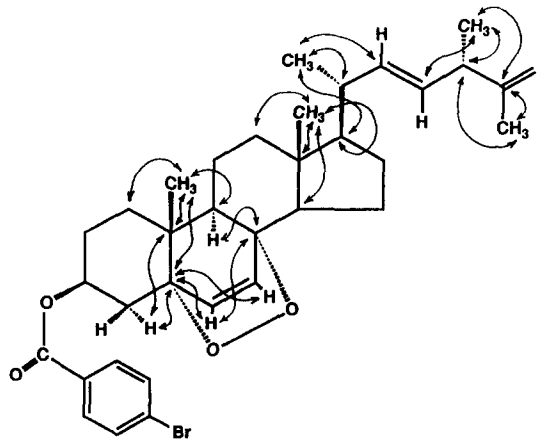


**Figure 2** $^{13}$C-$^1$H long-range correlations.

209–215 C, $[\alpha]_D$ +14.9°), whose physical properties were identical to those of **4**. Thus, the structure of axinysterol **(1)** was confirmed to be 5α,8α-epidioxyergosta-6,22,25-trien-3β-ol.

## Acknowledgment

The authors thank Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam, for identification of the sponge.

## References

1. Sheikh YM, Djerassi C (1974). Steroids from sponges. *Tetrahedron* **30:**4095–4103.
2. Gunatilaka AAL, Gopichand Y, Schmitz FJ, Djerassi C (1981). Minor and trace sterols in marine invertebrates. 26. Isolation and structure elucidation of nine new 5α,8α-epidioxy sterols from four marine organisms. *J Org Chem* **46:**3860–3866.
3. Findlay JA, Patil D (1984). A novel sterol peroxide from the sea anemone *Metridium senile*. *Steroids* **44:**261–265.
4. Jiménez C, Quiñoá E, Castedo L, Riguera R (1986). Epidioxy sterols from the tunicates *Dendrodoa grossularia* and *Ascidiella aspersa* and the gastropoda *Aplysia depilans* and *Aplysia punctata*. *J Nat Prod* **49:**905–909.
5. Miyamoto T, Honda M, Sugiyama S, Higuchi R, Komori T (1988). Isolation and structure of two 5α,8α-epidioxysterols and a cholesteryl ester mixture from the albumen gland of *Aplysia juliana*. *Ann Chem:* 589–592.
6. Jiménez C, Quiñoá E, Riguera R, Vilalta R, Quintela JM (1989). The Dietary origin of epidioxy steroids in *Actinia equina*. A carbon-14 incorporation experiment. *J Nat Prod* **52:**619–622.
7. Iguchi K, Kitade M, Yamada Y, Ichikawa A, Ohtani I, Kusumi K, Kakisawa H (1991). Stereostructures of unique 13-membered carbocyclic cembranolides from the soft coral *Lobophytum pauciflorum*. *Chem Lett* 319–322.
8. Iguchi K, Nishimura K, Yamazaki K, Iwashima M, Yamada Y (1992). New cembranolide diterpenes with a dimethylamino group from the Okinawan soft coral (*Sinularia* sp.). *Chem Lett* 127–130.
9. Iguchi K, Shimada Y, Yamada Y (1992). Hyrtiosal, a new sesterterpenoid with a novel carbon skeleton from the Okinawan marine sponge *Hyrtios erectus*. *J Org Chem* **57:**522–524.
10. Windaus A, Brunken J (1928). Uber die Photochemische Oxydation des Ergosterins. *Ann Chem* **460:**225–235.
11. Windaus A, Bergmann W, Luttringhaus A (1929). Uber Umsetsungen des Ergosterinperoxyds. *Ann Chem* **472:**195–201.
12. Clayton RB, Henbest HB, Jones ERH (1953). Studies in the steroid group. Part LX. Reduction of ergosterol epidioxide. *J Chem Soc* 2015–2021.